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EFFECTS OF EARLY AND LATE NEONATAL BROMOCRIPTINE TREATMENT ON HYPOTHALAMIC NEUROPEPTIDES, DOPAMINERGIC REWARD SYSTEM AND BEHAVIOR OF ADULT RATS

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Abstract—In humans, bromocriptine (BRO) is used as a treatment for many disorders, such as prolactinomas, even during pregnancy and lactation. Previously we demonstrated that maternal BRO treatment at the end of lactation programs offspring for obesity and several endocrine dysfunctions. Here, we studied the long-term effects of direct BRO injection in neonatal Wistar rats on their dopaminergic pathway, anxiety-like behavior and locomotor activity at adulthood. Male pups were either s.c. injected with BRO (0.1 μg/once daily) from postnatal day (PN) 1 to 10 or from PN11 to 20. Controls were injected with methanol–saline. Body mass, food intake, neuropeptides, dopamine pathway parameters, anxiety-like behavior and locomotor activity were analyzed. The dopamine pathway was analyzed in the ventral tegmental area (VTA), nucleus accumbens (NAc) and dorsal striatum (DS) at PN180. PN1–10 BRO-treated animals had normal body mass and adiposity but lower food intake and plasma prolactin (PRL). This group had higher POMC in the arcuate nucleus (ARC), higher tyrosine hydroxylase (TH) in the VTA, higher dopa decarboxylase (DDc), higher D2R and μu-opioid receptor in the NAc. Concerning behavior in elevated plus maze (EPM), BRO-treated animals displayed more anxiety-like behaviors. PN11–20 BRO-treated showed normal body mass and adiposity but higher food intake and plasma PRL. This group had lower POMC in the ARC, lower TH in the VTA and lower DAT in the NAc. BRO-treated animals showed less anxiety-like behaviors in the EPM. Thus, neonatal BRO injection, depending on the time of treatment, leads to different long-term dysfunctions in the dopaminergic reward system, food intake behavior and anxiety levels, findings that could be partially due to PRL and POMC changes.

Key words: lactation, programing, rat, dopamine, anxiety, locomotor activity.

INTRODUCTION

Dopamine is a neurotransmitter that mediates neurochemical, behavioral, endocrine and physiological functions (Sibley, 1999). Dopamine receptors are divided into two classes: D1 receptor (D1R and D5R) and D2 receptor (D2R, D3R and D4R). The D1R is expressed on postsynaptic neurons, modulate motor and cognitive functions and increases the incentive for acquiring natural rewards (Arnsten and Li, 2005; Chudasama and Robbins, 2006). The D2R is expressed on presynaptic and postsynaptic neurons, regulating motor function and behavior; it inhibits some hormones like prolactin (PRL) in the pituitary and, in the hypothalamus, is responsible for autonomic control (Missale et al., 1998).

Bromocriptine (BRO) is a D2R agonist and has a partial antagonist effect on the D1 receptor (Webster, 1996). BRO is used in the treatment of Parkison's disease (Seeman and Van, 1994), amenorrhea (Luboshitzky et al., 1980), prolactinoma tumoral growth (Gruszka et al., 2001), to restore fertility in hyperprolactinemic women (Krupp and Monka, 1987) and has been recently used in the treatment of type 2 diabetes (Liang et al., 2015). BRO is also used worldwide for suppressing lactation (Verma et al., 2006; Bernard et al., 2015). Besides, dopamine has been used in pediatric and neonatal intensive care units, particularly in the treatment of critically sick newborn infants (Noori and Seri, 2012; Saini et al., 2014).

Metabolic programing is a concept that relates events during the organism development with physiological changes that occur later in life, which are caused by epigenetic alterations that occurred during the original event (Waterland and Garza, 1999; Lucas, 2000; deMoura et al., 2008). This concept was confirmed by several epidemiological studies, which led to the Barker hypothesis of developmental origins of health and disease

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Abbreviations: ARC, arcuate nucleus; BRO, bromocriptine; CA, closed arms; CN, center; CON, control group; D1R, dopaminergic receptor 1; D2R, dopaminergic receptor 2; DDc, dopa decarboxylase; DS, dorsal striatum; EPM, elevated plus maze; NAc, nucleus accumbens; NPY, neuropeptide Y; OA, open arms; OF, open field; P, periphery; POMC, proopiomelanocortin; PRL, prolactin; TH, tyrosine hydroxylase; VTA, ventral tegmental area.

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(Barker et al., 1993). Lactation is a critical window for developmental plasticity (Gluckman and Hanson, 2007) and long-term changes in homeostatic metabolic, neural and hormonal regulation.

As previously described, BRO is used as a treatment for many disorders, such as pituitary tumor, even during pregnancy and lactation (Maeda et al., 1983; Raymond et al., 1985; Bronstein et al., 2002). Krupp and Monka (1987) collected data during the 8 years of BRO use in pregnancy, demonstrating that exposure to this drug in utero had no adverse influence on postnatal development. Shibli-Rahhal and Schlechte (2011) showed that children of mothers who used BRO during pregnancy had no abnormalities on physical and psychomotor development up to 9 years of age. More recently, additional studies have demonstrated the safety of BRO use (Lian et al., 2015; Almalki et al., 2015). However, there are no clinical or epidemiological studies evaluating the long-term programing effects of neonatal BRO treatment in the adult progeny. In addition, despite dopamine use in the treatment of shock in neonates, there is no report assessing its possible programing effects.

Our group has already used BRO-treatment in lactating rat dams to evaluate the programing effects of early weaning on offspring development. PRL inhibition at the end of lactation caused several changes in the adult offspring, such as: higher visceral fat mass (with adipocyte hypertrophy), hyperinsulinemia, leptin resistance, dyslipidemia, hypothyroidism and hypoprolactinemia (Bonomo et al., 2007, 2008; Moura et al., 2009; Peixoto-Silva et al., 2014) Higher neuropeptide Y (NPY) content and astrogliosis in hypothalamus (Younes-Rapozo et al., 2015) were also observed, as was higher levels of anxiety-like behavior and impaired learning/memory performance (Fraga et al., 2011).

The postnatal period in rodents roughly corresponds to the 3rd trimester of gestation in humans concerning neural development, depending on the structure that is being analyzed (Clancy et al., 2007; Nunes-Freitas et al., 2012; Dearden and Ozanne, 2015). The postnatal period spanning the first 10 days after birth are characterized by intense neural proliferation and synaptogenesis and, during this period, the number of DA fibers and both receptor types are higher than in adults. Besides, during this period an increase in μu opioid receptor occurs (Spain et al., 1985). Both μu opioid and dopamine pathways are important for the mesolimbic reward system and BRO stimulates these pathways (Azaryan et al., 1996; Gugusheff et al., 2015). The subsequent 10 days of life (from postnatal day 11 to 20) are still characterized by considerable synaptogenesis, gliogenesis and myelinization, as well as by the development of the hippocampus and of the reward pathways. Besides, during this period, D2R levels are higher than D1R ones, and still higher than at adulthood (Dow-Edwards et al., 1988, 1993; Hughes et al., 1993; Levitt, 1998; Tarazi and Baldessarini, 2000).

Since maternal BRO treatment at the end of lactation produces several programing effects in the adult offspring, and BRO is transferred through the milk (Katz et al., 1985), it is important to isolate the main imprinting factor: (1) it could be a direct BRO effect; (2) it could be an effect of a change in milk composition; or (3) it could be merely an effect of malnutrition. Thus, we decided to study the long-term effects on the dopamine pathway, on anxiety-like behavior and on locomotor activity, of BRO injection directly in rat pups at different times of the maturation of the dopaminergic system: at the beginning [from postnatal (PN) day to PN10] and at the end (from PN11 to PN20) of lactation. Our hypothesis is that direct treatment of the pups with BRO may affect the development of their dopaminergic system, leading to a sequence of programing effects that depend on the period of administration.

EXPERIMENTAL PROCEDURES

This study was conducted under the approval of the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro (CEUA/003/2014), which based its decision on the principles promulgated by Brazilian Law n° 11.794/2008. The experiments were performed to minimize the number of animals used and any suffering, following the ethical doctrine of the three ‘‘Rs” (reduction, refinement and replacement).

Wistar rats were housed in a temperature-controlled vivarium at (25 ± 1 °C) with artificial dark–light cycle (lights on from 7:00 a.m. to 7:00 p.m.). Female Wistar rats were mated with male rats at a proportion of 2:1. During pregnancy and lactation, the dams were housed in individual cages and had *ad libitum* access to water and a standard pellet diet (commercial control diets for rats, Labina, Purina®). After birth (defined as postnatal day 1, PN1), litters were culled to six male pups in order to improve the lactation performance.

Ten lactating dams were used and four male pups per litter were randomly chosen for the two experiments described bellow:

Early postnatal period programing (PN1–10)

At PN1, we randomly chose one pup from a given litter to receive s.c. bromo-α-ergocriptine (BRO – Novartis, São Paulo, Brazil) injections (once daily from PN1 to PN10; dose: 0.1 μg diluted in methanol–saline (1:1). This dose was calculated based on the dose administered to the mothers and that programed for some dysfunction in adult offspring (Bonomo et al., 2007, 2008; Moura et al., 2009). A second randomly chosen pup from the same litter received methanol–saline for the same period (CON). Experiment 1 BRO pups were identified by a non-toxic indelible ink mark on the front left paw, while Experiment 1 CON pups were marked on the front right paw.

Late postnatal period programing (PN11–20)

From PN11 to PN20, two more pups in a given litter underwent the same BRO and CON injection procedures indicated above. Experiment 2 BRO pups were identified by a non-toxic indelible ink mark on the hind left paw, while Experiment 1 CON pups were marked on the hind right paw.